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Nadi-Reaction and Cytochromes in the Pollen Grains of Some Higher Plants

By

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It has widely been accepted among biochemists that the substance which is responsible for the intracellular oxidation of p-phenylendiamine, is cytochrome oxidase. STOTZ, SIDWELL and HOGNESS (1938), however, have expressed the view that the oxidation of p-phenylendiamine is due not only to cytochrome c and its oxidase, but to cytochrome b, and OKUNUKI (1941) has arrived at a conclusion that cytochrome oxidase directly oxidazes, not only cytochrome c but p-phenylendiamine also. As a result of spectrographical and histochemical studies, AMANO (1939) has stated that cytochrome c oxidizes Nadi-reagent.

In the course of a histochemical study of *Lilium* anthers, it has been found that the sporogenous cells, that oxidize the Nadi-reagent, show characteristic cytochrome spectra (cf. SINKE, IIJIMA and HIRAOKA, 1947). The present study intends to find the relationship between the intracellular oxidation of the Nadi-reagent and the presence of cytochromes.

Results

1) Spectrographical and Cytochemical Studies of Living Pollen Grains.

Part of mature pollen grains collected was suspended in small amount of water and examined spectrographically with or without $Na_2S_2O_4$. At the same time, another part of the materials was tested with Nadi-reagent on a slide glass under a microscope. The results obtained are summarized in Table 1.

In Table 1, it is seen that the pollen grains we studied are divided, at least, into three types in respect to the presence or absence and the position of the absorption bands.

In the first type, the pollen grains give two distinct absorption bands lying at ca. 565 mμ and ca. 550 mμ, the latter being far more distinctly observed than the former in most cases. Frequently, a band is visible at ca. 605 mμ besides

Table 1

Plant Name	Nadi-reation (Cytoplasm)	Spectrogram		
		a (605 mμ)	b (565 mμ)	c (550 mμ)
Taxus cuspidata umbraculifera	—	—	—	—
Podocarpus macrophylla	+ or ±	—	++	
Abies firma	—			
Keteleeria Davidiana var. formosana	—	—	—	—
Pinus densiflora	±	—	—	—
P. densiflora var.	±	—	—	—
P. Thunbergii	±	—	—	—
P. taeda	±	—	—	—
P. Ban'siana	±	—	—	—
Sciadopitys verticillata	+	—	+	
Cryptomeria japonica	—	—	—	—
Cunninghamia lanceolata	+	—	—	—
Secale cereale	+	+?	+	++
Zea mays	+ or ++	+?	—	++
Trachycarpus excelsa	+	±	+	+
Lilium longiflorum	+ or ++	—	+	++
Crinum asiaticum	++	+	+	++
Fritillaria Thumbergii	+	+?	+	++
Narcissus Tazetta var. *chinensis*	+ or ++	±	+	+
Alstromeria sp.	+ or ++	—	—	++
Iris Pseudacorus	+	—	+	++
Platycarya strobilacea	+	±	+	+
Paeonia albiflora var. *hortensis*	+ or ++	+	+	++
Paeonia suffruticosa	+ or ++	+?	+	++
Nuphar japonicum	+	±	+	++
Podophyllum pleianthum	++	±	+	++
Magnolia liliflora	+	±	+	+

Magnolia denudata	+	±	++	++
Magnolia grandiflora	+	—	+	++
Camellia japonica	+	+?	+	+
Rosa multiflora	+	—	+	++
Althaea rosea	+	—	++	+?
Chaenomeles lagenaria	+	±	+	++
Punica Granatum	+	+	++	++
Oenothera odorata	+	—	—?	++
Campsis chinensis	+ or ++	+?	+	++
Cudrania triloba	+	±	+	++
Sarracenia sp.	+	+	+	++

the two distinct bands stated above. These three bands correspond to the characteristic absorption bands of cytochrome b, c and a, respectively. This fact as well as the statement of several workers that the cytochromes is contained widely in the cells of some higher plants support the view that the substances which are responsible for these absorption bands found in the present study are cytochromes (cf. KEILIN, 1925, OKUNUKI, 1939, YAKUSHIJI, 1935, GODDARD, 1944). *Crinum*, *Punica*, *Podophyllum*, *Chaenomeles* and others belong to this type.

In *Podocarpus* and *Sciadopitys* which belong to the second type there is only a single broad band lying between ca. 560 mμ and 555 mμ. TAMIYA and YAMAGUCHI (1933) have reported cytochromes in some bacteria that show characteristic bands lying at 561 mμ–557 mμ.

Most gymnospermous plants we studied, such as, *Pinus*, *Taxus*, *Cryptomeria*, *Cunninghamia* belong to the third type. In these plants the pollen grains show no sign of characteristic absorption bands of cytochromes with or without $Na_2S_2O_4$.

The results obtained in the test of the pollen grains with Nadi-reagent are given in Table 1. In this table, it is seen that the plants in which the cytoplasm of the pollen grains oxidizes the Nadi-reagents more or less strongly, show the characteristic absorption bands of cytochrome b and c and that those in which the cytoplasm is not or hardly coloured blue, the absorption bands are hardly recognized. It is probable, therefore, that positive Nadi-reaction carries intimate relation to the presence of cytochrome b and c.

It must be stated that many small granules which are colored blue with the Nadi-reagent were observed in the cytoplasm of the pollen grains.

2) Heat Experiment with Pollen Grains.

According to KEILIN (1929), cytochromes a′ and b′ are destructed while c′

remains unchanged even in boiling for several minutes. In the present investigation, the pollen grains of several plants were tested as in (1) after a preliminary heat treatment.

In this experiment, the pollen grains were heated in a glass vessel at 100°C for 15 minutes in a dry or moist state.

Results obtaind in this experiment are given in Table 2.

Table 2

Plant Name	Nadi-reaction		Cytochrome spectra					
	dry state	moist state	dry state a	dry state b	dry state c	moist state a	moist state b	moist state c
Podocarpus macrophylla	+ or ±	—						
Abies firme	—	—						
Keteleeria Davidiana var. *formosana*	—	—						
Pinus densiflora	±	—						
P. densiflora var.	±	—						
P. Thunbergii	±	—						
Sciadopitys oerticillata	+	—						
Narcissus Tazetta var. *chinensis*	+	—	−	+	+	—	—	—
Paeonia suffruticosa	+	—	−	−	±	—	—	—
Magnolia liliflora	+	—	−	+	+	—	—	—
Camellia japonica	+	—	−	+	+	—	—	—
Chaenomeles lagenaria	+	—	−	−	+	—	—	—

It is seen in this table, that when the pollen grains are heated in moist state, neither positive reaction color nor the cytochrome spectra are obtained, while the pollen grains heated in dry state show these characteristics as in the case of living state.

The granules in cytoplasm are coloured blue or violet with Nadi-reagent, even when pollen grains are heated in moist state. It is very probable to consider that this coloration of cytoplasmic granules is not due to direct action of oxidases but to after staining of the indophenol-blue.

3) Heating Experiment of Yeast and Wing Muscles of *Cicada*, *Bombus* and Honey Bees.

Yeast cells and wing muscles of some insects have been recommended by KEILIN (1929) as excellent materials for a spectrographical study of the cytochromes

in living cells. In the present experiment, Baker's yeast, wing muscles of *Cicada sp.*, *Bombus sp.* and honey bees were tested as in the case of the pollen grains given above, and results obtained in the wing muscles of *Cicada* are shown in Table 3.

Table 3

Pre-treatment	Nadi-reaction	Cytochrome spectra		
		a	b	c
None.	+++	++	++	+++
100°C-30 min. (dry state).	+	+	+	+++
100°C-30 min. (moist state).	—	—	—	++
100°C-1 hr. (dry state).	—	—	—	++
100°C-1 hr. (moist state).	—	—	—	++
70°C-30 min. (dry state).	++	++	++	+++
70°C-30 min. (most state).	± or —	+	+	++
Boiled-10 min.	—			

In Table 3, it is seen that when the muscles do not show positive Nadi-reaction, the absorption bands of cytochromes a and b are completely or almost invisible while the band of cytochrome c is visible more or less distinctly. The same results are obtained in yeast cells and wing muscles of *Bombus* and honey bees.

Conclusion

Results of our heating experiment of pollen grains, yeast cells and wing muscles of insects, suggest that cytochrome a appears to carry no important relation with the oxidation of Nadi-reagent, since the Nadi-reaction is positive while the absorption band of cytochrome a is absent in the pollen grain of several plants given in Table 1. Our tentative conclusion is therefore, that the substance* which play an important rôle in oxidation of the Nadi-reagent in the living cells, is destructed by heat treatment and probably be a cytochrome oxidase or cytochrome b.

* The view preliminarily expressed in a previous paper that this substance was thermolabile cytochrome c, is abandoned. (SINKE, IIJIMA and HIRAOKA, 1947).

Summary

Pollen grains of some angiospermous plants have cytochromes while those of most gymnospermous plants we studied do not show cytochrome spectra.

There are intimate relations between the positive Nadi-reaction and the presence of cytochrome b in pollen grains, yeast cells and wing muscles of some insects.

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